

The Chemical Form of Cadmium in Subcellular Fractions Following Cadmium Exposure

by Keizo Waku*

Wistar rats were given drinking water containing 250 ppm Cd for 12 months. After excision of the kidney and liver, the organs were subfractionated into nuclear, mitochondrial, microsomal and cytosol fractions, and the chemical forms of Cd in the subcellular fractions were examined. Although approximately 90% of the total Cd was present in the cytosol, in the form of metallothionein, 3-5% was also present in the mitochondrial fraction and 5-7% in the microsomal fraction from both organs. By Sephadex G-75 gel filtration, after solubilizing the particulate fractions with sodium deoxycholate, approximately 89% of Cd in the microsomal fraction and 94% in the mitochondrial fraction eluted with the same retention time as that of metallothionein in both liver and kidney, while the remainder was found in a high molecular weight protein fraction. The Cd that eluted with the high molecular weight protein fraction may be involved in dysfunction in subcellular organelles.

For estimating the toxicity of Cd associated with the high molecular weight fraction, rat liver microsomes and CdCl₂ were mixed with 1% of sodium deoxycholate and the protein-Cd complex produced was isolated by eluting with Sephadex G-75. This complex had a strong toxicity toward the alcohol dehydrogenase activity (SH-enzyme), and the *K_i* values of the Cd-protein complexes decreased with increased amount of Cd bound to the microsomal protein fraction. The above results suggest that loosely bound Cd increased in the case of higher Cd/protein and plays a toxic role in the living cells.

Introduction

Cd is recognized as a highly toxic environmental contaminant (1) and can cause damage to several organs, particularly renal dysfunction (1). Furthermore, it has been suggested that Itai-Itai disease may be due to oral intake of Cd from the Jintsu river in Japan (2).

Several observations have indicated preferential accumulation of Cd in liver and kidney when animals received this metal (1, 3). Most of the Cd thus accumulated in the organs is bound to metallothionein located in the cytosol (4, 5). Recent studies have shown that the metallothionein may have a protective role against Cd poisoning (6-9).

On the other hand, electron microscopic studies have described inducement, by Cd administration, of morphological alterations of the subcellular organelles of kidney and liver of experimental animals (10-12). In addition, it has been reported

that oxidative phosphorylation by isolated mitochondria (13) and protein synthesis by isolated ribosomes (14) were depressed in Cd-fed rats. This suggests that the Cd, as located in subcellular organelles, may be a primary location for the subsequent toxic effect in tissues. However, it has not been demonstrated how Cd is associated with subcellular organelles.

The present investigation was first undertaken to reveal the chemical forms of Cd in microsomal and mitochondrial fractions of rat liver and kidney after long-term exposure of Cd. The results show that over 90% of Cd in the two particulate fractions is bound to a low molecular weight material corresponding to metallothionein, and that the remainder is associated with high molecular weight proteins.

Second, for the estimation of toxicity of Cd associated with high molecular weight proteins, the complex of rat liver microsomes and Cd was prepared and estimated on the inhibitory action toward the alcohol dehydrogenase activity. This

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complex has a lower K_i value when a relatively higher amount of Cd is associated with the microsomes.

Materials and Methods

Ten male Wistar rats, weighing 220–250 g, were allowed free access to water containing 250 ppm Cd as cadmium chloride. Ten control rats were given Cd-free drinking water. All animals were killed by decapitation after 12 months exposure. The kidney and liver were quickly excised and weighed.

The tissue was homogenized in a Potter-Elvehjem homogenizer with 0.25 M sucrose. The tissue homogenate (10%) was subfractionated into nuclear (which also contained cell debris), mitochondrial, microsomal, and cytosol fractions by differential centrifugation. The supernatant from the nuclear pellet (600g, 5 min) was centrifuged at 11,000g for 10 min to obtain the mitochondrial fraction. The microsomal and cytosol fractions were obtained from the postmitochondrial supernatant by centrifugation at 105,000g for 60 min. The nuclear fraction was obtained by using Hitachi refrigerated centrifuge Model 05PR-2. Microsomal and mitochondrial fractions were separated in a Hitachi ultracentrifuge Model 65P-7 by using an RP50-2 rotor. Each pellet from the differential centrifugation was resuspended in 0.25 M sucrose, and this resuspension was centrifuged again under each given condition. The washing procedure was repeated three times. A portion of each fraction was fixed overnight in 2.5% glutaraldehyde and postfixed for 2 hr in 1% OsO_4 . Then the purity of each preparation was checked by microscopic examination. Tissue and subcellu-

lar fractions for Cd assay were subjected to nitric acid digestion, and then assayed for Cd by an atomic absorption procedure with a Perkin-Elmer Model 703 spectrophotometer. Protein concentrations were assayed by the method of Lowry et al. (15).

The mitochondrial and microsomal preparations were solubilized with 1% sodium deoxycholate (DOC) (Difco Laboratories, U.S.A.) by stirring for 15 min in an ice-cold bath under an N_2 stream. Each solution was adjusted to contain 1.5% protein. In some cases, 1% sodium dodecyl sulfate (SDS) (Wako Pure Chemical Industries, Japan) or 4% Triton X-100 (Wako Pure Chemical Industries) was used in the place of DOC. Each solubilized fraction or supernatant fraction was applied to a column (2.6 \times 67 cm) of Sephadex G-75. The column was eluted with 10 mM Tris-HCl buffer, pH 7.5, containing 0.1% DOC. To avoid artificial changes in Cd-containing materials, the fractionating procedures were performed at 4°C within 24 hr after excising the tissues. A portion of each fraction (5 mL) was assayed for absorbance at 250 nm, and Cd was estimated by atomic absorption spectroscopy.

Preparations of Cd-Microsomal Protein Complexes

A 3 g portion of rat liver was homogenized with 12 mL of 1.15% KCl-10 mM Tris-HCl buffer (pH 7.5) in a Teflon homogenizer and centrifuged (12,000g) for 20 min. The supernatant was centrifuged (105,000g) again for 1 hr, and precipitated microsomal proteins (100 mg protein) were dissolved in 1% DOC to make up to 3 mL. Several quantities of CdCl_2 solution (4.5×10^{-2} M) were

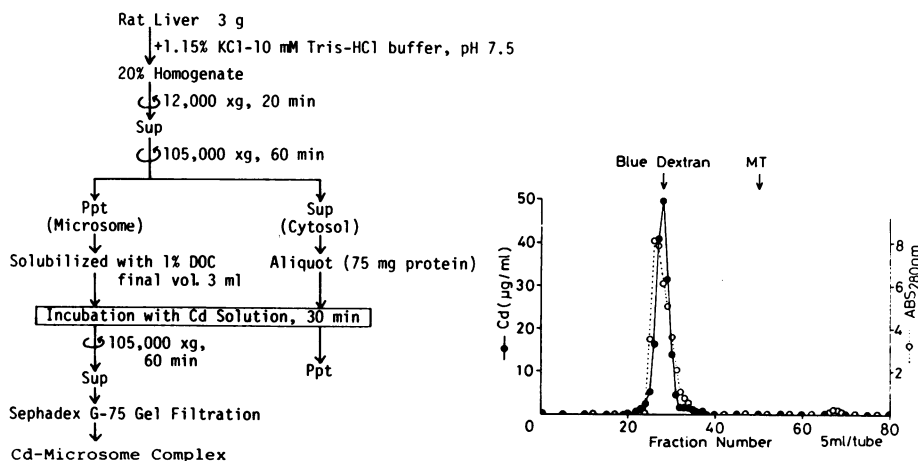


FIGURE 1. Preparation of Cd-microsomal fraction complex and separation of Cd-microsome complex by Sephadex G-75.

Table 1. Estimation of alcohol dehydrogenase (ADH) activity.^a

Incubation mixture	Microsomes	Cd complex	Cd ion + microsomes
0.2 M Tris-HCl buffer, pH 8.5, mL	0.2	0.2	0.2
NAD (1.25–10.0 mM), mL	0.1	0.1	0.1
5.14 M Ethanol, mL	0.1	0.1	0.1
Microsomal fraction (1 mg protein), mL	0.5	—	0.455
Cd-complex fraction (1 mg protein), mL	—	0.5	—
CdCl ₂ solution, mL	—	—	0.045
Yeast ADH (37.5 µg/mL), mL	0.1	0.1	0.1
Total	1.0 (mL)	1.0 (mL)	1.0 (mL)

^aFrom estimated increase of ABS_{340nm} (25°C).

added to the solubilized microsomal solution (0.15–1.2 mg Cd) and stirred for 30 min at room temperature. After centrifugation for 30 min (105,000g), the supernatant was applied to Sephadex G-75 gel filtration (flow rate 4 mL/10 min) and Cd-microsome complex was eluted on void volume (Fig. 1).

Assay of Alcohol Dehydrogenase Activity

Alcohol dehydrogenase activity (E.C. 1111) was estimated spectrophotometrically by measuring the initial ratio of the reduction of NAD in the presence of excess ethanol (Table 1). In this procedure, the reaction mixture consisted of 0.1 mL of a solution containing several quantities of NAD in distilled water, 0.2 mL of Tris-HCl buffer (pH 8.5), 0.1 mL of ethanol and 0.5 mL of a solution containing several quantities of CdCl₂-microsome complex. Just before the measurement, 0.1 mL of alcohol dehydrogenase (yeast, Boehringer Mannheim, West Germany) water suspension (37.5 µg/mL) was added. The absorbance of the mixture at 340 nm was estimated at intervals of 10 sec from zero time. As a control experiment, 0.5 mL of distilled water with same quantity of microsomes was added instead of Cd-microsome complex. Furthermore, we estimated the inhibitory effect of adding CdCl₂ and microsomes added separately, to the cuvette. In this case, most of Cd acts as a free Cd ion to alcohol dehydrogenase activity.

Results

The amount of Cd taken was on the average 17.2 mg/kg body weight/day in the experimental rats. Growth of Cd-fed rats was significantly retarded within a month and continuously thereafter (Fig. 2). The average body weight of Cd-fed rats was approximately 60% of that of control rats after 12 months exposure. The weight of liver or kidney as percentage of total body weight did not change with Cd intake.

Figure 3 shows electron micrographs of mito-

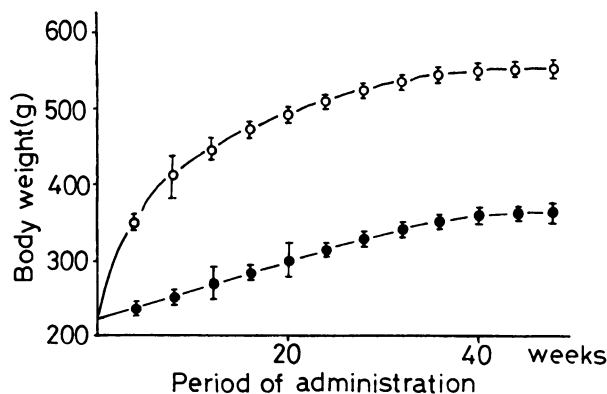


FIGURE 2. Effect of CdCl₂ administration on rat body weight. Experimental rats were given drinking water containing 250 ppm CdCl₂; (○) control rats (no Cd); (●) experimental rats. All animals were weighed at the indicated time. Body weight was the mean of 10 rats.

chondria and microsomal fractions of the liver from the Cd-fed rat. Damage to subcellular organelles by Cd could not be clearly observed after fractionation. The mitochondrial fraction contained both mitochondria and lysosomes, and endoplasmic reticulum; nuclear and cell debris were not found. On the other hand, the microsomal fraction consisted of fragments of rough and smooth endoplasmic reticulum while neither mitochondria nor lysosomes were present. The electron microscopic appearance of each particulate fraction from kidney was similar to that of liver (results not shown). Distribution of marker enzymes could not be determined because the activities of several marker enzymes, e.g., glucose-6-phosphatase, were found to be greatly lowered after Cd administration.

The estimated distribution of Cd in the organ subcellular fractions is summarized in Table 2. Although the Cd content of the cytosol was significantly greater than in the other subcellular fractions obtained from both kidney and liver, 3–5% of Cd was found in the mitochondrial fraction and 5–7% in the microsomal fraction of both organs. These particulate fractions were washed three

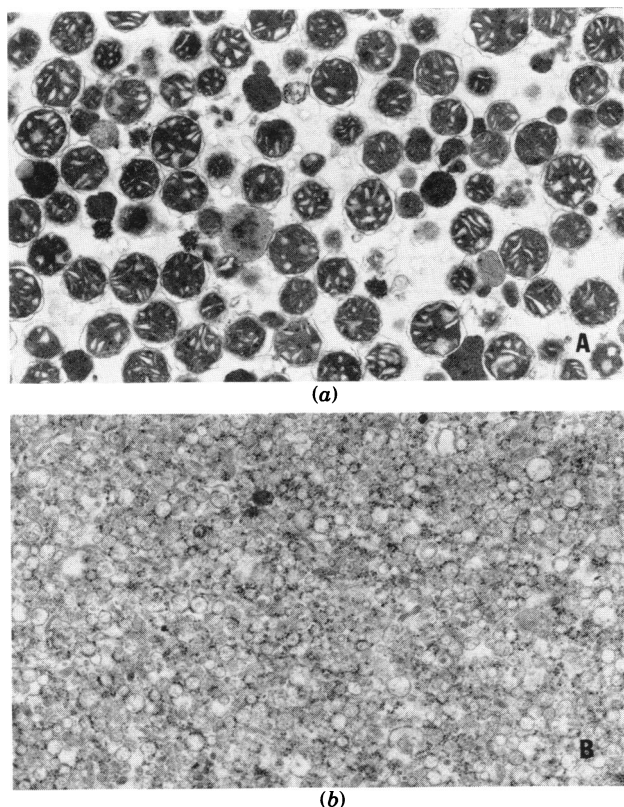


FIGURE 3. Electron micrographs of (a) mitochondrial fraction ($\times 8,568$) and (b) microsomal fraction ($\times 12,495$) (rat liver). The rat was given drinking water containing 250 ppm CdCl_2 for 12 months.

times by resuspension and recentrifugation before Cd assay, but the additional washing procedure did not change the ratio of Cd per protein. For example, in one preparation of microsomal fraction of liver, the ratios were 0.093, 0.090 and 0.087 $\mu\text{g}/\text{mg}$ protein after the first, second and third washing, respectively, and 0.077 $\mu\text{g}/\text{mg}$ protein after an additional washing. For the same corresponding operations in the washing procedure, the ratios of the quantity of Cd in the

supernatant to that of Cd in precipitate were 25.42, 0.39, 0.33 and 0.07, respectively. These results demonstrate that the Cd in each particulate fraction was not derived from the cytosol.

To solubilize the microsomal and mitochondrial fractions, three detergents, DOC, SDS and Triton X-100, were used. Over 95% of the total protein was solubilized by 1% DOC or 1% SDS, while 4% Triton X-100 solubilized only 44% of the total protein of the microsomal fraction and 89% of the mitochondrial fraction. Moreover, to estimate the effect of detergents on metallothionein, DOC or SDS was added to the cytosol of liver from Cd-fed rats to make a 1% solution of each detergent, and then each solution was applied to a column of Sephadex G-75. When SDS was added, 4–5% of the total Cd was shifted from the metallothionein fraction to the void volume, whereas this was not observed with DOC. Since these results indicated that DOC was most appropriate to solubilize the subcellular particles, 1% DOC was used in the subsequent experiments.

Figure 4 shows representative profiles of Sephadex G-75 gel filtration of the solubilized subcellular fractions. Over 98% of Cd in cytosol from kidney or liver was found to be bound to a low molecular weight material. This material corresponded to metallothionein as reported by Kägi and Vallee (16). The chromatographs of both the microsomal and the mitochondrial fractions from both organs were the same as those of cytosol, except that there was another Cd-containing peak of a high molecular weight eluted at the void volume. The percentage of Cd eluted at the void volume was found to be $11.1 \pm 0.6\%$ (mean \pm SD, $n = 4$) in the microsomal fraction, and $6.1 \pm 1.2\%$ ($n = 4$) in the mitochondrial fraction from both kidney and liver. On the other hand, in the cytosol from both organs, only $1.0 \pm 0.2\%$ ($n = 4$) was eluted at the void volume.

For characterization of the low molecular weight Cd binding material in the microsomal

Table 2. Subcellular distribution of Cd in rat liver and kidney after oral administration of 250 ppm CdCl_2 for 12 months.

Fraction	Distribution of Cd ^a			
	Liver, % of total in homogenate ($\mu\text{g}/\text{mg}$ protein)		Kidney, % of total in homogenate ($\mu\text{g}/\text{mg}$ protein)	
Nuclei ^b	0.77 ± 0.13	(0.051 ± 0.011)	1.58 ± 0.32	(0.139 ± 0.028)
Mitochondria ^c	3.61 ± 0.22	(0.092 ± 0.018)	4.65 ± 0.37	(0.034 ± 0.010)
Microsome	5.54 ± 1.62	(0.090 ± 0.004)	7.13 ± 0.66	(0.337 ± 0.021)
Cytosol	91.60 ± 2.98	(1.661 ± 0.161)	87.52 ± 0.43	(2.310 ± 0.416)

^aMean \pm SD for 10 experiments.

^bThis fraction contained nuclei and cell debris.

^cThis fraction contained mitochondria and lysosomes.

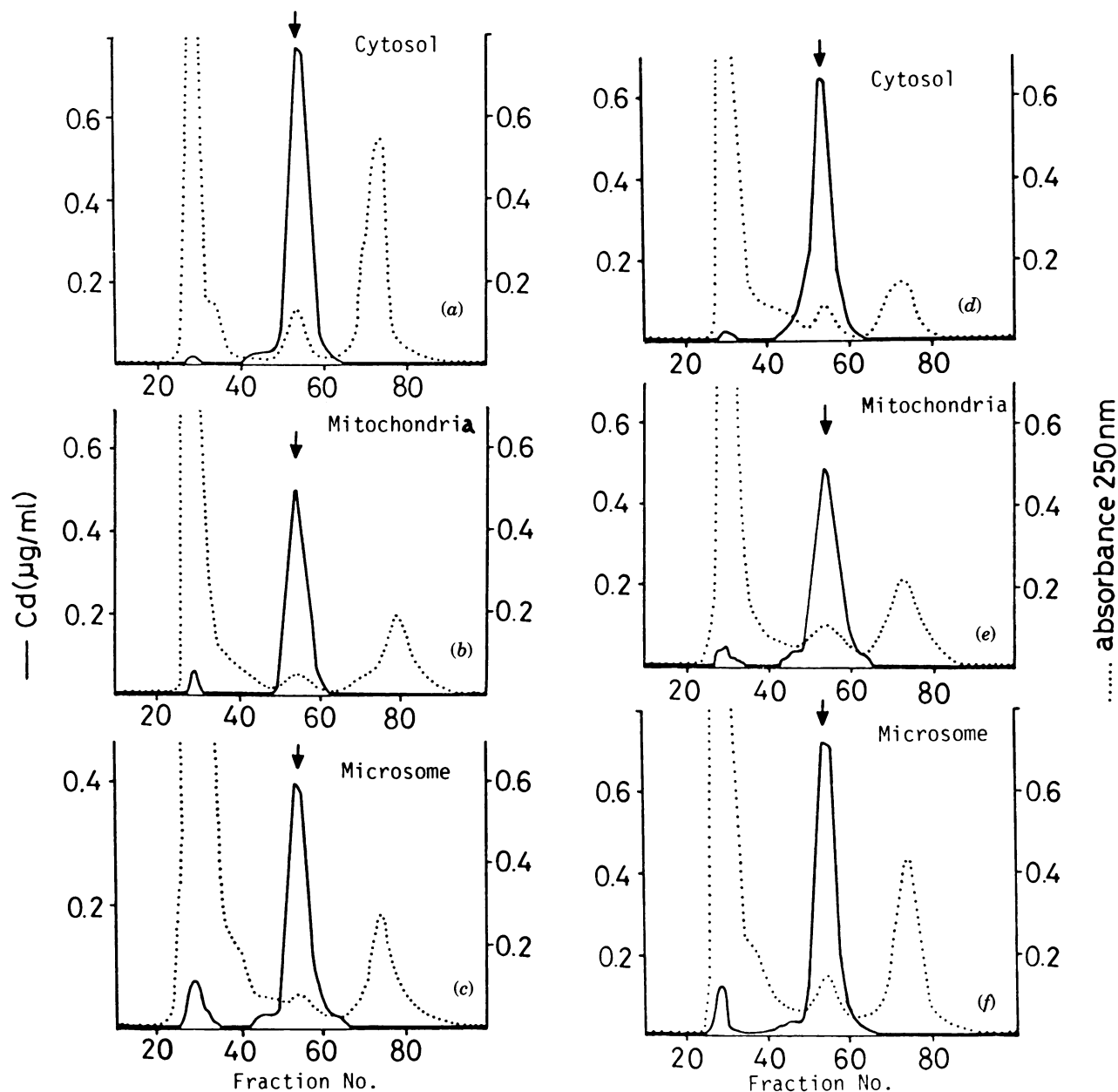


FIGURE 4. Sephadex G-75 separation of cytosol (a,d), mitochondrial (b,e) and microsomal (c,f) fractions from rat liver (a-c) and kidney (d-f) after oral administration of 250 ppm CdCl_2 for 12 months. Arrow indicates the position of cytochrome c eluted.

and mitochondrial fractions, ultraviolet spectra were taken (data not shown). Each absorption spectrum had a shoulder at 250 nm, which was lost by acid treatment at pH 2.0. This finding implied that Cd was bound by SH groups in this material.

Several Cd-protein complexes were prepared with various concentrations of Cd with the same quantities of microsomes. As shown in Figure 1, Cd-microsome complex was eluted at void vol-

ume. This Cd-microsomal complex is composed of protein and lipid at the same ratio of those of original rat liver microsomes. The Cd-protein ratio of the complex with respect to quantity of Cd added is shown in Figure 5. If we assume that average molecular weight of the protein eluted at void volume is 100,000, the Cd/protein molar ratio is ca. 4 when 0.4 mg Cd was added to 75 mg protein. For estimating the degree of inhibitory effects of several ratios of the Cd-protein com-

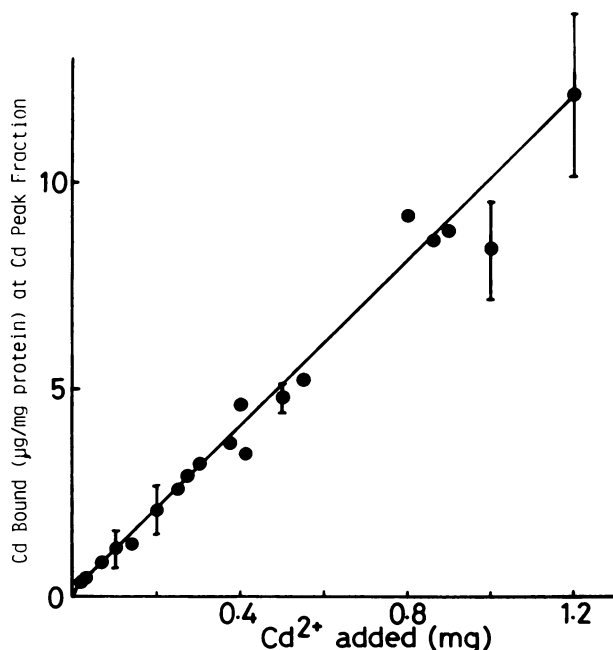


FIGURE 5. Binding of cadmium to the solubilized microsomal component as a function of quantity of Cd added.

plex, the alcohol dehydrogenase activity was estimated at the various ratios of Cd in the complexes. As a control, experiments were carried out on the inhibitory effects of adding free ion and microsomes separately or microsomes only (Fig. 6). In the case of small amount of Cd associated with the same quantity of microsomal protein (Fig. 6a), the inhibitory curve of the Cd-microsome complex is very close to the activity curve of control experiments (added microsomes only). In the case of higher amounts of Cd associated to protein (Figs. 6 b,c,d), however, the inhibitory curve approaches more closely the inhibition curve of the corresponding free Cd ion with the increase of Cd/protein ratio.

To determine the modes of inhibition of alcohol dehydrogenase activities by various concentra-

tions of Cd associated with the certain amount of microsomal proteins, Lineweaver-Burk plots were prepared. The tested complexes included 3.28×10^{-6} to 7.16×10^{-5} M Cd as a final concentration in the cuvette. It was found that noncompetitive type inhibition occurred in each case. The results obtained are shown in Table 3. The K_i and V_{max} values calculated for the inhibitors are summarized. It can be seen that, when Cd ion and microsomes were added separately, K_i values are the same at the different Cd concentrations, while different concentrations of Cd associated with the microsomes have different K_i values. In the case of adding higher amounts of Cd associated with microsomes, the K_i value becomes closer to that of free Cd ion + microsomes.

Discussion

The data in this paper show that, in liver and kidney of chronically Cd-fed rats, most of the Cd was located in the cytosol as previously described (17-20). There was, however, 5-7% Cd in the microsomes and 3-5% in the mitochondria and lysosomes. The possibility that the particulate fractions were contaminated by cytosol was eliminated because the Cd content per protein of these fractions did not change after extensive washing. Since several authors have reported morphological changes of subcellular organelles (10-12) and dysfunction of mitochondria or ribosomes (13,14), then any Cd located in the mitochondria and microsomes may be expected to be involved in the toxic effect.

Over 98% of the Cd in the cytosol was eluted with metallothionein by Sephadex G-75 gel filtration. Sato and Nagai (21) reported that, except for metallothionein, one to four additional Cd-containing elution fractions were found in the cytosol of liver or kidney after subcutaneous administration of Cd. Under our experimental conditions, these fractions were not found in the cytosol of the organs.

Gel filtration of extracts of the subcellular par-

Table 3. V_{max} and K_i values of Cd-complex or Cd ion + microsomes with alcohol dehydrogenase activity.

Cd concn in incubation mixture, M	Cd-microsome complex			Cd ion + microsomes		
	K_m , mM	V_{max} , µmole/min	K_i , M	K_m , mM	V_{max} , µmole/min	K_i , M
0	0.625	1.000	—			
3.28×10^{-6}	0.625	0.952	6.56×10^{-5}	0.625	0.833	1.64×10^{-5}
4.04×10^{-6}	0.625	0.935	5.77×10^{-5}	0.625	0.800	1.62×10^{-5}
7.47×10^{-6}	0.625	0.833	3.74×10^{-5}	0.625	0.685	1.62×10^{-5}
1.92×10^{-5}	0.625	0.549	2.34×10^{-5}	0.625	0.459	1.63×10^{-5}
2.95×10^{-5}	0.625	0.455	2.46×10^{-5}	0.625	0.357	1.64×10^{-5}
7.16×10^{-5}	0.625	0.250	2.39×10^{-5}	0.625	0.235	1.68×10^{-5}

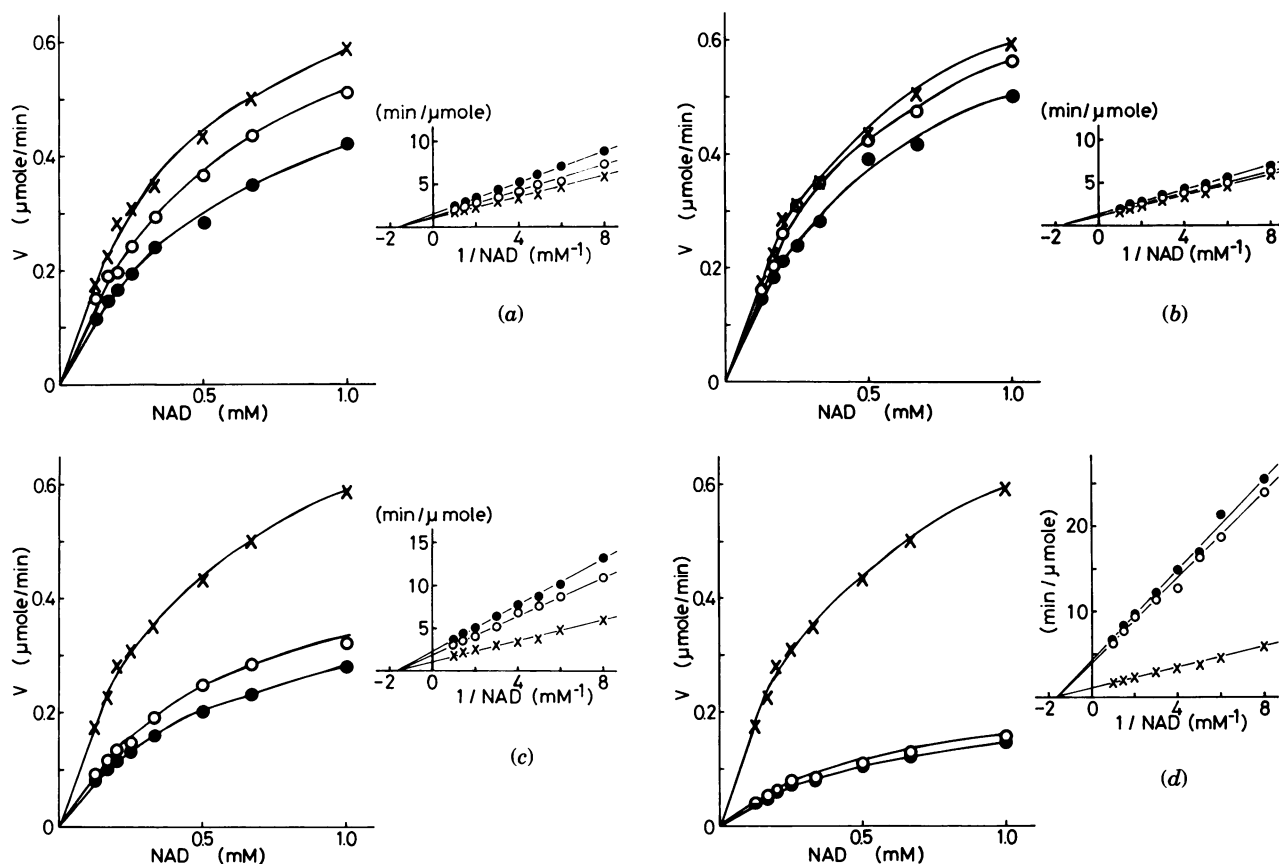


FIGURE 6. Inhibition of ADH activity by Cd-microsome complex; (—x—) microsomes only; (—○—) Cd-microsome complex; (—●—) Cd + microsomes at various Cd concentrations; (a) 3.28×10^{-6} M, (b) 7.47×10^{-6} M, (c) 1.92×10^{-5} M, (d) 7.16×10^{-5} M. Cd-microsome complex was prepared with (a) 15 μg Cd^{2+} (b) 70 μg , (c) 110 μg , (d) 860 μg , and fractionated by Sephadex G-75 as shown in Fig. 1.

ticles showed that about 89% of the Cd in the microsomal fraction and 94% in the mitochondrial fraction was found to be bound to a low molecular weight material in both kidney and liver. This Cd binding material is identical with metallothionein in the following properties: (a) the elution volume for this material was about two times of the void volume of the column (16), and this peak exactly coincided in position with metallothionein in the cytosol; (b) the absorption spectrum of this fraction had a shoulder at 250 nm corresponding to Cd bound to SH groups of protein. Sato and Nagai (22) also isolated the same fraction from a rat liver particulate fraction (27,000g, 60 min). Further investigations are required to determine whether this material is completely identical with the metallothionein of the cytosol.

The present study also showed the existence of other Cd-binding material of high molecular weight in each particulate fraction. The possibility that this material may be the aggregate of

metallothionein is unlikely because the fractionation was performed at 4°C within 24 hr after excising the tissues to avoid the denaturation of the protein and also because this peak was not found in the chromatograph of cytosol even when DOC was added to the cytosol fraction.

Several recent reports have suggested that metallothionein may have a protective effect against Cd toxicity (6–9). Furthermore, Waku et al. (23) found that metallothionein had no inhibitory effect on the activity of the microsomal enzyme, acyltransferase, whose activity was inhibited by Cd. This indicates that the Cd eluted in the high molecular weight fraction may relate to dysfunctions of subcellular organelles. A similar suggestion has been presented by Kimura et al. (7) from the examination of Cd toxicity on bone.

In the next step, we studied the toxic effect of the high molecular weight material associated with Cd. We attempted to form complexes of proteins such as bovine serum albumin, γ -globulin, cytosol protein or microsomal protein with Cd.

However, cytosol protein formed a precipitate with CdCl_2 (0.1–1.2 mg Cd/75 mg protein). Bovine serum albumin did not precipitate with Cd ion, but could not form a complex with Cd. (Cd was eluted in a position of free Cd ion on Sephadex G-75 column). Only rat liver microsomal protein complexed with Cd. This may be due to the fact that 28% of the microsomes are composed of phospholipid (24), because Cd could form a complex with phosphatidylcholine (25) and may help to form a complex with Cd. As shown in Figure 5, Cd ion could associate with up to 12 molecules/molecules microsomal protein, if we assume that the molecular weight of microsomal protein is 100,000. As shown in Table 3, K_i values and V_{\max} became smaller when more quantities of Cd were associated with the same quantities of microsomes. Especially, at Cd concentration of 3.28×10^{-6} to 7.47×10^{-6} M, the K_i values are higher than those of the same amounts of free Cd ion, and at more than 1.92×10^{-5} M, the K_i values show the same figures (2.3 or 2.4×10^{-5} M) and are close to that of free Cd ion (1.64×10^{-5} M). The above results suggest that Cd could associate with the microsomes rather tightly when a smaller amount of Cd is associated with the microsomes, and if more quantities of Cd were associated, the loose binding of Cd increases and may play a more toxic role in the living cells.

As shown by Waku et al. (23), at the level of chronic administration of Cd ion into the rat, ca. 250 $\mu\text{g/g}$ tissue of Cd accumulated in the kidney or 2×10^{-3} M in the tissue. If we assume that 1% of Cd is located at the high molecular weight material, ca. 2×10^{-5} M Cd associated with this material. Therefore, as shown in Table 3, this concentration of Cd could be loosely bound to the high molecular weight material and could act as a toxic substance in the cells.

REFERENCES

1. Friberg, L., Piscator, M., Nordberg, G. F., and Kjellström, T. In: *Cadmium in the Environment*, 2nd ed. CRC Press, Cleveland, 1974, pp. 9–21, 101–114.
2. Tsuchiya, K. Causation of Ouch-Ouch disease (Itai-Itai Byo). An introductory review. *Keio J. Med.* 18: 181–194 (1969).
3. Lucis, O. J., Lynk, M. E., and Lucis, R. Turnover of cadmium 109 in rats. *Arch. Environ. Health* 18: 307–310 (1969).
4. Shaikh, Z. A., and Lucis, O. J. Induction of cadmium-binding protein (abstract). *Fed. Proc.* 29: 298 (1970).
5. Shaikh, Z. A., and Lucis, O. J. Cadmium and zinc binding in mammalian liver and kidneys. *Arch. Environ. Health* 24: 419–425 (1972).
6. Suzuki, Y., and Yoshikawa, H. Role of metallothionein in the liver in protection against cadmium toxicity. *Ind. Health* 12: 141–151 (1974).
7. Kimura, M., Otaki, N., Yoshiki, S., Suzuki, M., and Horiuchi, N. The isolation of metallothionein and its protective role in cadmium poisoning. *Arch. Biochem. Biophys.* 165: 340–348 (1974).
8. Hildebrand, C. E., Tobey, R. A., Campbell, E. W., and Enger, M. D. A cadmium-resistant variant of the Chinese hamster (CHO) cell with increased metallothionein induction capacity. *Exptl. Cell Res.* 124: 237–246 (1979).
9. Tsuno, H., Nakajima, H., Hata, A., and Kimura, M. Genetic influence on induction of metallothionein and mortality from cadmium intoxication. *Toxicol. Letters* 4: 253–256 (1979).
10. Nishizumi, M. Electron microscopic study of cadmium nephrotoxicity in the rat. *Arch. Environ. Health* 24: 215–225 (1972).
11. Kimura, M. Acute cadmium poisoning. 1. Electron microscopic study of acute cadmium poisoning rat liver. *Ochanomizu Med. J.* 21: 1–21 (1973).
12. Fowler, B. A., Jones, H. S., Brown, H. W., and Haseman, J. K. The morphologic effects of chronic cadmium administration on the renal vasculature of rats given or normal calcium diet. *Toxicol. Appl. Pharmacol.* 34: 233–252 (1975).
13. Sato, N., Kamada, T., Suematsu, T., Shichiri, M., Hayashi, N., Matsumura, T., Kishida, Y., Abe, H., Furuyama, F., Ishibashi, F., and Hagihara, B. Cadmium toxicity and liver mitochondria. 1. Different effect of cadmium in vitro to adult, young and ethionin-fed rats. *J. Biochem. (Tokyo)* 84: 117–125 (1978).
14. Gamulin, S., Car, M., and Narancsik, P. Effect of cadmium on polyribosome structure and function in mouse liver. *Experientia* 33: 1144–1145 (1977).
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin reagent. *J. Biol. Chem.* 193: 265–275 (1951).
16. Kägi, J. H., and Vallee, B. L. Metallothionein: a cadmium- and zinc-containing protein from equine renal cortex. *J. Biol. Chem.* 235: 3460–3465 (1960).
17. Webb, M., and Daniel, M. Induced synthesis of metallothionein by pig kidney cells in vitro in response to cadmium. *Chem. Biol. Interact.* 10: 269–276 (1975).
18. Cherian, M. G., Goyer, R. A., and Delaquerriere-Richardson, L. Cadmium-metallothionein-induced nephropathy. *Toxicol. Appl. Pharmacol.* 38: 399–408 (1976).
19. Ando, M., Sayato, Y., and Tonomura, M. Hygienic chemical study on poisonous metal. IV. Researches on behavior of intestinal absorption into living organisms. *J. Hyg. Chem.* 22: 24–30 (1976).
20. Squibb, K. S., and Cousins, R. J. Synthesis of metallothionein in a polysomal cell-free system. *Biochem. Biophys. Res. Commun.* 75: 806–812 (1977).
21. Sato, M., and Nagai, Y. Mode of existence of cadmium in rat liver and kidney after prolonged subcutaneous administration. *Toxicol. Appl. Pharmacol.* 54: 90–99 (1980).
22. Sato, M., and Nagai, Y. Cadmium in rat liver particulate fraction. *J. Hyg. Chem.* 26: 103–105 (1980).
23. Waku, K., Hayakawa, F., and Nakazawa, Y. The effect of cadmium ions and cadmium-metallothionein on the activities of phospholipid synthesizing enzymes of rat liver microsomes in vitro. *Arch. Biochem. Biophys.* 204: 288–393 (1980).
24. Cook, R. P. Distribution of sterols in organs and in tissue. In: *Cholesterol*. Academic Press, New York, 1958, pp. 145–180.
25. Faure, M. Method for purification of lecithins. *Bull. Soc. Chim. Biol.* 32: 503–508 (1950).